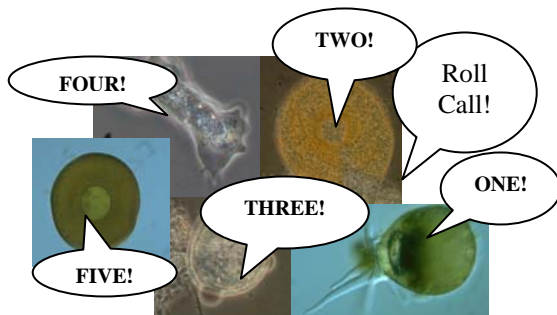


PROTOZOAN COUNTS



*By Toni Glymph, Wastewater Microbiologist
Metropolitan Water Reclamation District of
Greater Chicago*

As the environment in the aeration basin changes one type of microorganism is dominated by another. The microorganism best suited for the environment will emerge until the environment changes again. Changes in pH, dissolved oxygen, temperature, nutrients, competition etc., all determine which species will dominate. The protozoan species that are most dominant in the treatment system indicate which conditions are most dominant. Although, protozoan species dominance should not be relied on solely, to troubleshoot wastewater treatment conditions, this information is very helpful in assessing the conditions of the activated sludge process.

This protozoan count procedure is not designed to determine the total number of each type of protozoan that is present in the system. Instead, what is important is the relative numbers of one type in comparison to another type. In other words, the purpose is to determine which species seems to be dominating. The count will examine protozoa in the following categories:

- Amoebae
- Flagellates
- Free-swimming ciliates
- Crawling ciliates
- Stalked ciliates

- Metazoa (rotifers, nematodes, water bears etc.)
- Shelled/Testate species

In a well-operating system, the ciliates will most likely be the dominant species. Ciliates dominate when most of the nutrients have been removed from the wastewater. In a poorly operated system, amoebae and flagellates will probably dominate. Amoebae and flagellates can only compete for dominance when there are still plenty of nutrients remaining. On the other hand stalked ciliates and Metazoa will be predominant in longer age systems mainly because of their ability to compete when very little nutrients are left and their ability to feed on other protozoa.

The Procedure

Collect a fresh, well-mixed, representative sample of mixed liquor from the discharge end of the aeration basin. If the concentration of the mixed liquor is quite high, the sample should be diluted. If you choose to dilute the sample, always use the same dilution if you plan to do routine counts. Ideally, the sample should be observed under the 20X objective using the phase contrast condenser. Using a smaller objective (10X or below) will make it difficult to see the smaller flagellates and using a larger objective (40X or higher) will narrow your field of view.

1. Place one drop of mixed liquor on a clean grease-free slide and cover with a cover slip.
2. Scan the slide using 5 passes. Focus the objective at the top left corner of the cover slip. Moving down the cover slip, count and record the number of each type of protozoa that you see. This completes one pass. Move over to the right slightly and this time move up the cover slip keeping a running total the number of each type of protozoa. This

will complete the second pass. Move up and down the slide until you have completed 5 passes.

3. Record the running total number of each protozoa type. Remember, if you encounter colonies of stalked ciliates you must count (or estimate) the total number of heads. The motto is, “The more heads, the older the sludge”. For the best results, scan 3 or more slides and average the number of each protozoan type.
4. Calculate relative dominance
 - a. Count and record the total number of protozoa in each category. Record the counts from all three slides (See example Count Worksheet).
 - b. Next, calculate the average number of each organism from all three slides.
 - c. Calculate the percentage by dividing the average number of each organism by the total number of microorganisms counted and multiply by 100.
 - d. Group amoebae and flagellates together and combine the percentage. Note rather or not the amoeba are “naked” or “testate”
 - e. Group all the ciliates together and combine the percentage and calculate the percentage of Metazoa.
5. Amoebae and flagellates are grouped together because they are both indicators of young sludge or incomplete treatment. In a well-operated system, ciliates should dominate. Higher numbers of Metazoa are associated with longer sludge ages.
6. Calculate protozoan count per milligram of mixed liquor
7. To determine the count/mg ML, divide the protozoan count by the MLVSS.

Protozoan counts are not exact science and should not be relied on solely, for determining treatment

system conditions. They are helpful however, in helping the operator to assess conditions within the aeration basin.

Example Count Worksheet

ORGANISM	<i>Slide #1</i>	<i>Slide #2</i>	<i>Slide #3</i>	<i>Average</i>	<i>Percent</i>
Amoeba	6	3	1	3	4%
Flagellate	13	6	6	8	10.5%
Free-swimming ciliates	15	12	7	11	14.5%
Crawling ciliates	22	15	20	19	25%
Stalked ciliates	40	32	23	32	42%
Metazoa (Rotifers, nematodes etc.)	5	1	2	3	4%
Shelled/Testate species					
<i>Totals</i>	101	69	59	76	